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(54) Title: AUTOMATED MOLECULAR BIOLOGICAL DIAGNOSTIC SYSTEM

(57) Abstract

Self-addressable, self-assembling microelectronic system for performing molecular diagnosis, analysis, multi-step and multiplex reactions in microscopic formats. Actively controlled reactions include nucleic acid hybridization, immunoassays, clinical diagnosis and multi-step combinatorial biopolymer synthesis. Controller interfaces with user via input/output devices preferably including a graphical display. The controller may interface with a power supply and interface, the interface providing selective connection to individual microlocations, polarity reversal, and selective potential or current levels to individual electrodes. A combined system for performing DNA sample preparation, hybridization, detection and data analysis integrates multiple steps. Charged materials are transported preferably by free field electrophoresis. DNA complexity reduction is preferably achieved by binding DNA to a support, cleaving unbound materials such as by restriction enzymes, and transporting the cleaved fragments. Active, programmable matrix devices include a square matrix pattern with fanned out electrical connections and optional electrical connections beneath specific microlocations resulting in a highly automated DNA diagnostic system.

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DESCRIPTION

Automated Molecular Biological Diagnostic System

Field of the Invention

This invention relates to devices and systems for
5 performing multi-step molecular biological type diagnostic
analyses in multiplex formats. More particularly, the
molecular biological type analyses include various nucleic
acid hybridizations reactions and associated biopolymer
synthesis. Additionally, antibody/antigen reactions and
10 other clinical diagnostics can be performed.

Related Application Information

This application is a continuation-in-part of appli-
cation Serial No. 08/271,882, filed July 7, 1994, which is
a continuation-in-part of Serial No. 07/146,504,
15 filed November 1, 1993, both entitled "SELF-ADDRESSABLE
SELF-ASSEMBLING MICROELECTRIC SYSTEMS AND DEVICES FOR
MOLECULAR BIOLOGICAL ANALYSIS AND DIAGNOSTICS."

Background of the Invention

Molecular biology comprises a wide variety of
20 techniques for the analysis of nucleic acid and protein.
Many of these techniques and procedures form the basis of
clinical diagnostic assays and tests. These techniques
include nucleic acid hybridization analysis, restriction
enzyme analysis, genetic sequence analysis, and the
25 separation and purification of nucleic acids and proteins
(See, e.g., J. Sambrook, E. F. Fritsch, and T. Maniatis,
Molecular Cloning: A Laboratory Manual, 2 Ed., Cold
spring Harbor Laboratory Press, Cold Spring Harbor, New
York, 1989).

30   Most of these techniques involve carrying out numer-
ous operations (e.g., pipetting, centrifugations, electro-
phoresis)on a large number of samples. They are often
complex and time consuming, and generally require a high
degree of accuracy. Many a technique is limited in its

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actual hybridization reaction is performed. Finally, detection and data analysis convert the hybridization event into an analytical result.

The steps of sample preparation and processing have
5 typically been performed separate and apart from the other main steps of hybridization and detection and analysis. Indeed, the various substeps comprising sample preparation and DNA processing have often been performed as a discrete operation separate and apart from the other substeps.
10 Considering these substeps in more detail, samples have been obtained through any number of means, such as obtaining of full blood, tissue, or other biological fluid samples. In the case of blood, the sample is processed to remove red blood cells and retain the desired nucleated
15 (white) cells. This process is usually carried out by density gradient centrifugation. Cell disruption or lysis is then carried out, preferably by the technique of sonication, freeze/thawing, or by addition of lysing reagents. Crude DNA is then separated from the cellular
20 debris by a centrifugation step. Prior to hybridization, double-stranded DNA is denatured into single-stranded form. Denaturation of the double-stranded DNA has generally been performed by the techniques involving heating ($>Tm$), changing salt concentration, addition of base
25 (NaOH), or denaturing reagents (urea, formamide, etc.). Workers have suggested denaturing DNA into its single-stranded form in an electrochemical cell. The theory is stated to be that there is electron transfer to the DNA at the interface of an electrode, which effectively weakens
30 the double-stranded structure and results in separation of the strands. See, generally, Stanley, "DNA Denaturation by an Electric Potential", U.K. patent application 2,247,889 published March 18, 1992.

Nucleic acid hybridization analysis generally in-
35 volves the detection of a very small number of specific target nucleic acids (DNA or RNA) with an excess of probe DNA, among a relatively large amount of complex non-target

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July 8, 1987) and for the detection of overlapping clones
and the construction of genomic maps (G. A. Evans, in US
Patent Number 5,219,726, June 15, 1993).

5   New techniques are being developed for carrying out
multiple sample nucleic acid hybridization analysis on
micro-formatted multiplex or matrix devices (e.g., DNA
chips) (see M. Barinaga, 253 Science, pp. 1489, 1991; W.
Bains, 10 Bio/Technology, pp. 757-758, 1992). These
methods usually attach specific DNA sequences to very
10   small specific areas of a solid support, such as micro-
wells of a DNA chip. These hybridization formats are
micro-scale versions of the conventional "dot blot" and
"sandwich" hybridization systems.

        The micro-formatted hybridization can be used to
15   carry out "sequencing by hybridization" (SBH) (see M.
Barinaga, 253 Science, pp. 1489, 1991; W. Bains, 10
Bio/Technology, pp. 757-758, 1992). SBH makes use of all
possible n-nucleotide oligomers (n-mers) to identify n-
mers in an unknown DNA sample, which are subsequently
20   aligned by algorithm analysis to produce the DNA sequence
(R. Drmanac and R. Crkvenjakov, Yugoslav Patent Applica-
tion #570/87, 1987; R. Drmanac et al., 4 Genomics, 114,
1989; Strezoska et al., 88 Proc. Natl. Acad. Sci. USA
10089, 1992; and R. Dramanac and R. B. Crkvenjakov, U.S.
25   Patent #5,202,231, April 13, 1993).

        There are two formats for carrying out SBH. The
first format involves creating an array of all possible n-
mers on a support, which is then hybridized with the
target sequence. The second format involves attaching the
30   target sequence to a support, which is sequentially probed
with all possible n-mers. Both formats have the fundamen-
tal problems of direct probe hybridizations and additional
difficulties related to multiplex hybridizations.

        Southern, United Kingdom Patent Application GB
35   8810400, 1988; E. M. Southern et al., 13 Genomics 1008,
1992, proposed using the first format to analyze or se-
quence DNA. Southern identified a known single point

Beattie et al., in The 1992 San Diego Conference: Genetic Recognition, November, 1992, used a microrobotic system to deposit micro-droplets containing specific DNA sequences into individual microfabricated sample wells on a glass substrate.

Generally, the prior art processes have been extremely labor and time intensive. For example, the PCR amplification process is time consuming and adds cost to the diagnostic assay. Multiple steps requiring human intervention either during the process or between processes is suboptimal in that there is a possibility of contamination and operator error. Further, the use of multiple machines or complicated robotic systems for performing the individual processes is often prohibitive except for the largest laboratories, both in terms of the expense and physical space requirements.

As is apparent from the preceding discussion, numerous attempts have been made to provide effective techniques to conduct multi-step, multiplex molecular biological reactions. However, for the reasons stated above, these techniques are "piece-meal" and limited. These various approaches are not easily combined to form a system which can carry out a complete DNA diagnostic assay. Despite the long-recognized need for such a system, no satisfactory solution has been proposed previously.

Summary of the Invention

The present invention relates to the design, fabrication, and uses of a self-addressable self-assembling microelectronic devices and systems which can actively carry out controlled multi-step processing and multiplex reactions in a microscopic formats. These reactions include, but are not limited to, most molecular biological procedures, such as nucleic acid hybridization, antibody/antigen reaction, and related clinical diagnostics. In addition, the claimed devices and systems are able to carry out multi-step combinational biopolymer synthesis,

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and analysis. In this fullest form, the sample is first prepared, such as by an electronic cell sorter component. Generally, electronic refers more specifically to the ability of the component device to electrophoretically

5   transport charged entities to or from itself. Further DNA processing and complexity reduction may optionally be performed by a crude DNA selector component, and a re- striction fragment selector component. The final pro- cessed target DNA is transported to the analytical compo-

10   nent where electronic hybridization analysis is carried out in a microscopic multiplex format. This analytical component device is also referred to as the APEX or analytical chip. Associated detection and image analysis components provide the results.

15   Within the system materials may optionally be transported between components (devices) by free field electrophore- sis, channelling, fluidics or other techniques. Optional- ly, electronic reagent dispenser components can provide electrophoretic transport of reagents to the various

20   processing components of the system. Optionally, an elec- tronic waste disposal system may be formed by providing an electrode and charged matrix material that attracts and holds charged waste products. Optionally, an electronic DNA fragment storage system can serve to temporarialy hold

25   other DNA fragments for later hybridization analysis.

       In one aspect of this invention, genomic DNA complex- ity reduction is performed by processes that isolate those specific DNA fragments containing the desired target se- quence from the bulk of the DNA material that lacks the

30   desired target sequence. Crude DNA can be transported and captured on a support material. The bound DNA can then be severed using appropriate restriction enzymes. After severing, the DNA fragments can be transported to a component device that selectively hybridizes specific DNA

35   fragments. Those fragments that contain the actual target sequences to be analyzed can be selectively released, via further restriction enzyme cleavage, and transported to

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and magnifies the image from the hybridization events occurring on the various micro-locations of the device. The emissions are optically filtered and detected by a charge coupled device (CCD) array or microchannel plate
5   detector. The image is then stored and analyzed. Preferably, the results are displayed to the user on the monitor.

In another aspect of this invention, the hybridization system is formed having a plurality of microlocati-
10   ons formed atop a substrate containing control electronics. Specifically, switching circuits are provided to address individually the microlocations. The electrical connections are made via the backside relative to where sample contact is to be made. Additionally, an optical
15   pathway, such as a waveguide, is disposed beneath the microlocation to permit backside access to the microlocation. Optical excitation, if necessary, may be directed to the microlocation via the waveguide. Detection of emitted radiation may be detected via the backside wave-
20   guide. In yet another aspect of this invention, a sample containment system is disposed over the system, particularly the hybridization matrix region. In the preferred embodiment, the matrix hybridization region (including sample containment component) is adapted for
25   removal from the remainder of the device providing the electronic control and detector elements.

In another aspect of this invention, improved processes for forming a matrix hybridization system are described. In one process, a substrate, such as sili-
30   con, is formed with an insulating layer, such as a thick oxide. Conductive microlocations are formed, such as by deposition of metal (e.g., aluminum or gold) that is then patterned, such as by conventional photolithographic techniques. An insulating coating is formed, such as
35   TEOS formed by PECVD. Optionally, a nitride passivation coating is formed over the TEOS layer. Openings to the microelectrode are formed through the nitride and glass.

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Fig. 6 shows a plan view of the system including an electronic cell sorter matrix, DNA selectors and restriction fragment selectors and hybridization matrix.

Fig. 7 shows a block diagram description of the
5 control system.

Fig. 8 is a cross-sectional view of the active, programmable matrix system having associated electronics.

Fig. 9 is a cross-sectional view of an alternative,
10 layered active, programmable matrix system having electrical and optical access to the backside of the microlocations and a biological containment cover.

Fig. 10 shows a perspective view of the APEX system mounted in a mating carrier.

15 Figs. 11A-G show process steps in device fabrication.

Fig. 12 shows a fabricated device utilizing a polysilicon structure in cross-section.

Fig. 13 shows a fabricated device utilizing adhe-
20 sion enhancing layers in cross-section.

Fig. 14 shows a device having an enlarged reservoir space above the electrode.

Fig. 15 shows user displays for various voltage and current regimes.

25 Fig. 16 shows a cross-sectional view of a DNA purification system.

Fig. 17 shows a cross-sectional view of a capillary array manufacturing system and apparatus.

Fig. 18 shows a perspective view of a micro-loca-
30 tion of a concentric structure.

Detailed Description of the Invention

Figs. 2A and 2B illustrate a simplified version of the active programmable electronic matrix hybridization system for use with this invention. Generally, a sub-
35 strate 10 supports a matrix or array of electronically addressable microlocations 12. For ease of explanation,

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contacts the attachment layer 16A as a result of its
movement under the electrophoretic force, the functiona-
lized specific binding entity 20 becomes covalently
attached to the attachment layer 16A.

5   It is possible to protect the attachment layers
which are not subject to reaction, such as 16B and 16C
by making their corresponding electrodes 12B and 12C
negative.   This results in electrophoretic lines of
force emanating from the attachment region 16B (only 16B
10   will be discussed for simplicity, the results being
similar for 16C). The electrophoretic force lines 24
serve to drive away negatively charged binding entities
20 from the attachment layer 16B and towards the attach-
ment layer 16A. In this way, a "force field" protection
15   is formed around the attachment layers 16 which it is
desired to have nonreactive with the charged molecules
20 at that time.

One highly advantageous result of this system is
that charged binding materials 20 may be highly concen-
20   trated in regions adjacent to signal attachment layers
16. As can be seen in perspective drawing Fig. 2B, if a
individual microlocation 26A is positively charged, and
the remaining microlocation are negatively charged, the
lines of electrophoretic force will cause transport of
25   the net negatively charged binding entities 20 toward
the microlocation 26A. The microlocation 26A is intend-
ed to depict the combination in Fig. 2A of the attach-
ment layer 16, the permeation layer 14 and the underly-
ing associated electrode 12. In this way, a method for
30   concentrating and reacting analytes or reactants at any
specific microlocation on the device may be achieved.
After the attachment of the specific binding entities 20
to the attachment layer 16, the underlying microelectro-
de 12 may continue to function in a direct current (DC)
35   mode. This unique feature allows relatively dilute
charged analytes or reactant molecules free in solution
to be rapidly transported, concentrated, and reacted in

way, the concentration aspect is utilized to provide
high concentrations at that specific attachment layer
then subject to the positive electrophoretic force. The
concentrated materials may next be moved to an adjacent,

5   or other, attachment layer 16. Alternatively, multiple
attachment layers 16 may be deprotected in the sense
that there is a net electrophoretic force field emanat-
ing from the electrode 12 through the attachment layer
16 out into the reservoir 18. By deprotecting multiple

10   attachment layer 16, multiplex reactions are performed.
Each individual site 26 may serve in essence as a sepa-
rate biological "test tube" in that the particular envi-
ronment addressed by a given attachment layer 16 may
differ from those environments surrounding the other at-

15   tachment layers 16.

Fig. 3 shows a plan view of the metal mask layer
for an active programmable electronic matrix system. A
plurality of individual electrodes 30 are formed prefer-
ably in an array. For example, an 8 x 8 matrix of indi-

20   vidual electrodes 30 is formed. Optionally, additional
control or dump pads 32 may be provided to aid in gener-
ation of desired electrophoretic fields. The electrodes
30 and pad 32 are connected to contact pads 34. 68
contact pads 34 are shown corresponding to the 64 elec-

25   trodes 30 and 4 pads 32. Leads 36 connect the elec-
trodes 30 and pads 32 individually to the contacts 34.
As shown, a fan-out pattern is used to permit connec-
tions from the relatively condensed region of the elec-
trodes 30 and pads 32 to the boundaries 36 of the mask.

30   Fig. 4 shows an exploded detail plan view of the
mask of Fig. 3. The resulting metallized system would
appear substantially similar to the masked pattern. The
electrodes 30 are shown formed as substantially square
structures. The lead lines 36 connect the electrode 30

35   to the contact pad 34 (Fig. 3). The preferred line
width of the lead 36 is 1 to 20 microns.

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groups oe sets of locations can be made selective for
one type of cell. Cell selectivity can be imparted by
attaching specific antibodies or cell adhesion factors
to the attachment layer. The matrix 66 operates by free
5   field electrophoresis.

The crude DNA selector 68 and restriction fragment
selector 70 serve to bind the crude DNA output from the
electronic cell sorter matrix 66 and permit selective
cleavage of the desired DNA from the bound material.
10   The term crude is used merely to denote a non-final
stage in DNA isolation or complexity reduction. The DNA
is bound to the selector in a region which is believed
not to contain the desired DNA material. The desired
DNA materials are then severed from the bound materials,
15   such as by application of restriction enzymes. The
severed, unbound material is then physically moved from
the crude DNA selector 68 to the restriction fragment
selector 70. Preferably, electrophoretic transport is
used to remove the severed material. This process may be
20   repeated by binding the severed material to a selector,
upon which a restriction enzyme acts so as to cleave the
unbound portion which contains the desired DNA.

For example, human DNA contains approximately 100,-
000 genes. Of the total DNA material, a significant
25   portion constitutes repeating sequences which do not
contain the desired DNA information. The DNA may be
bound to a selector by these noninformation bearing
repeating sequences. The bound DNA may be severed from
the unbound DNA which is believed to contain the desired
30   DNA to be analyzed. This process may then be repeated
with yet more specific sequences causing binding of the
material to the selector.

The output of the restriction fragment selector 70
is then supplied to the APEX chip 72. Operations on the
35   matrix 72 are performed as described in connection with
Figs. 2A and 2B.

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nating or pulsed current at the strip electrode. This
drives DNA on and off of the stationary phase.

A further alternative method for reducing the com-
plexity of a sample of DNA, is to size select by sieving
5   the sample through a microporous media. Microporous
media can be formed by filling cavities of arbitrary
geometry with dendrites. These dendrites are formed by
electrochemical deposition of chemicals such as, but not
exclusive to, metal salts, ceramic forming materials,
10   monomers and polymers. The porosity of the microporous
media can be controlled by adjusting the electrical
signal that is applied to the electrodes. For example,
dendrites can form picket fence type structures or frac-
tal type structures.

15   A method for forming microporous media on an APEX
device could involve forming a long channel with oppos-
ing metal electrodes. When this channel is filled with
the appropriate chemical and an appropriate electrical
signal is applied to the electrodes, dendrites will form
20   in the interstitial space between the electrodes forming
a microporous media.

Returning to Fig. 6, an electronic reagent dispens-
er system 74 may be provided to deliver reagents to the
system 60. Preferably, the reagents are delivered by
25   electrophoretic force if they are charged. Optionally,
an electronic waste disposal system 76 is included with-
in the system 60. The waste disposal system 76 attracts
charged waste particles to it and disposes of them by
holding the charged entities on it. Another optional
30   member of system 60 is the DNA fragment storage system
78. This fragment storage system 78 serves to temporar-
illy hold DNA fragments for future analysis..

The system 60 may include some or all of the func-
tions described above. For example, the combination of
35   sample preparation in the form of complexity reduction,
as performed by the DNA selector 68 and restriction
fragment selector 70 may be associated with the analyti-

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Additionally, another relay permits selecting the polar-
ity of the voltages supplied to the APEX system 92 elec-
trodes. Optionally, if multiple source levels are avail-
able, such as from a multiple output power supply 86,

5   the specific level to be connected to an APEX system 92
electrode may be set independently of those for the
other electrodes.

Thus, as described in connection with Fig. 2A, by
placing certain electrodes (e.g., 12B and 12C) at a

10   negative, but lesser potential than electrode 12D, the
attachment region 16B and 16C would be protected by the
local force field.

The interface 88 may serve to select the desired
voltage for the individual electrodes in the APEX system

15   92. Alternatively, such a different voltage arrangement
may be achieved through use of a voltage divider.

In the preferred embodiment, the controller comput-
er 80 is a Macintosh Quadra 950. National Instruments
Corporation LabVIEW software is used to provide a soft-

20   ware interface for a user to program the devices con-
nected to the APEX and to collect and process data from
an assay. National Instruments NuBus boards are used to
provide the hardware interface from the Quadra 950 com-
puter 80 to the power supply devices 86 that source

25   potentials and currents and that measure the actual
currents and potentials and the results of the assay.

The user controls the assay through a Virtual In-
strument created with the LabVIEW software. The virtual
instrument provides a user friendly graphical represen-

30   tation of the controls that the user may exercise, and
of some of the results of applying these controls to the
APEX device to perform an assay. The user interfaces
with the Virtual Instrument through the keyboard and
mouse (collectively, input 84) of the Quadra 950 comput-

35   er 80. The Virtual Instrument provides software inter-
faces to a National Instruments NB-MIO-16XL multipurpose
input/output 90 and to a National Instruments DMA2800

ments SCXI Chassis with nine 16-channel, Class 3 Relay
Modules connected in the chassis, providing a total of
144 relays. Two relays are used per electrode to pro-
vide for electrode disconnected or electrode connected
5   to either positive or negative source. In the preferred
embodiment, a bundle of cables connects these relays to
the APEX device through a Cerprobe Probe Card that pro-
vides mechanical contact of probes to the bond pads of
the APEX device.

10   The controller computer 80 optionally controls the
illumination source 94 for excitation of fluorescence to
detect DNA hybridization. In the preferred embodiment,
the illumination source 94 is a laser which outputs
radiation at an appropriate wavelength to excite fluo-
15   rescent markers included within the APEX system 92.

The output of the APEX system 92 is passed through
observation path 96 to the detector 98. The observation
path 96 may be a physical connection, such as through a
fiber optic, or may comprise an optical path such as
20   through a microscope. Optical filters may be utilized
in the observation path to reduce illumination of the
detector at wavelengths not corresponding to the emis-
sion spectra of the fluorescent markers in the APEX
system 92. Additionally, notch filters may be utilized
25   as necessary to reduce illumination of the detector 98
at the excitation wavelength of the laser illumination
source 94. The detector 98 may optionally form an image
of the APEX system 92, such as through the use of a
cooled CCD camera. In addition to, or as an alterative
30   to, forming an optical image, the emitted fluorescence
radiation from the APEX system 92 may be detected by
conventional means such as photodiodes or photomultipli-
er tubes. The output of the detector 98 is provided to
the data processing/analysis system 100. This system
35   monitors the level of detected probe material in the
APEX system 92. Optionally, an expert system may be uti-
lized in the analysis system 100.

provide convenient activation paths for the individual
circuit elements 114.

    .   Waveguides can be used for guiding excitation light
to micro-locations, and for guiding fluorescence signals
5 to detectors. Waveguides can be free standing, as in an
optical fiber, or can be integrated into a monolithic
semiconductor device. Waveguides can be fabricated from
materials such as zinc oxide or indium tin oxide that
are also electrically conductive. The waveguide can
10 then serve as both an electrode and as means for trans-
porting optical radiation. Waveguides can be located in
or around the plane of the capture probe to minimize
nonspecific background fluorescence. Waveguides can
incorporate holographic optical elements. The function
15 of these holographic optical elements includes, but is
not exclusive to, notch filters, dichroic mirrors, band
pass filters, beam splitters, neutral density filters,
half-wave plates, quarter-wave plates, polarizers, and
lenses.

20   Fig. 9 shows in cross-section an alternative, lay-
ered structure for the active programmable matrix sys-
tem. In a first layer, individual electrodes 120 are
formed upon a support 122. The support 122 is prefer-
ably insulating. Above the electrodes 120 is preferably
25 formed a permeation layer 124 and individual attachment
layers 126 corresponding to the individual electrodes
120. Optical paths 128 are provided through the support
122 to access the electrode 120. Preferably, the opti-
cal path 128 is comprised of a fiber optic or light
30 guiding pipe or structure. Optionally, electrical con-
nection 130 passes through the support 122 to access the
electrodes 120 from the backside. The term backside is
used herein to connote that side of the electrode 120
which contacts the support 122. In a second layer a
35 semiconductor support 132 includes circuit elements 133
connected to the conductor 131. The conductor 131 is
designed such that its upper surface mates and forms

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Active, programmable matrices of micro-locations
can also be formed from capillary tubes. Fig. 17 shows
a system and product formed therefrom. Capillary tube
matrices are formed by stacking capillary tubes in ar-
5   rays 220 of arbitrary geometry, or by melting by heater
222 and drawing, such as by die 224, these arrays 220
into an adherent and integral unit 226. Alternatively,
solid rods composed of two diffenent materials arranged
about each other concentrically can be used instead of
10   capillary tubes. Fig. 18 shows such a structure in
perspective. Here, the material that composes the inner
core 230 is etched out from the outer material 234 se-
lectively to form a hole 232 that goes partially or all
the way through the device. Alternatively, the inner
15   core may be etched in such a way as to form a controlled
porousity glass.
Individual capillary tubes can be addressed by
wires inserted into the capillary tube, or by affixing
the capillary tube matrix to a complimentary matrix of
20   lithographically formed electrodes. Additionally, the
inner cores of the solid rods may be formed from a con-
ducting material. Electrical contact can be made with
the inner core material by affixing the solid rod matrix
to a complimentary matrix of electrodes, or by litho-
25   graphically forming electrodes on the solid rod matrix.
The capillary tubes and etched solid rods are
filled with an appropriate material to form a permeation
layer. The surface of the permeation layer can be func-
tionalized with specialized attachment chemistry.
30   An alternative method to electrophoretic transport
is to use convective mass transport to transport materi-
al to microlocations. One device that can accomplish
this is a rotating disk. Here convection is acheived by
the hydrodynamic shear forces present at boundary be-
35   tween the spinning disk and the solution. Fluid flows
straight onto the surface from the bulk solution. A
matrix of electrode pads can be attached to a spinning

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layer 156. The sequence of preferred steps is as fol-
lows. First, the semiconductor, preferably p-type, test
grade silicon is oxidized with a thick (10,000 Å) oxide.
A conductive polysilicon layer, preferably a polysilicon

5   doped 5,000 Å thick layer is formed. The polysilicon is
then patterned, preferably by photolithography using a
wet etch. Next, a glass layer, such as PECVD deposited
TEOS is formed. A layer approximately 3,000 Å formed at
475°C is preferred for improved adhesion. The glass

10 layer is then patterned, again, preferably with photoli-
thographic techniques using a wet etch. A metal layer
is then formed over the surface, preferably by sputter-
ing aluminum to a thickness of 3,000 Å. The metal is
then patterned, again preferably photolithographically,

15 with a wet etch. Next, a nitride layer is formed, pref-
erably via PECVD at 70°C to a thickness of 3,000 Å.
Next a via is formed photolithographically using a wet
etch so as to contact the electrode.

        Fig. 13 shows a structure having improved adhesion

20 of metal conductor to underlying insulating layers
through the use of an intermediate adhesive metal such
as titanium tungsten. A semiconductor 170, preferably
silicon, has disposed thereon an oxide layer 172. An
intermediate electrode layer 176, formed of a conductive

25 metal such as gold or aluminum, is sandwiched between
titanium tungsten 174 and 178. Adhesive metal layer 178
contacts the external electrode 184, preferably formed
of platinum. A glass layer 180, such as formed from
TEOS, underlies a external nitride coating 182.

30   Fig. 14 shows a cross-sectional view of an improved
electrode arrangement. An electrode 190 is disposed
adjacent to an insulator 192, preferably an oxide. A
nitride layer 194 overlies the insulator layer 192.
Preferably, the nitride layer 194 is undercut such that

35 the insulating layer 192 is set back from the edge 198
of the nitride layer. A reservoir 196 is thereby de-

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was mixed into the acrylamide/copolymer formulation to
provide a means for attaching specialized functionality
to the surface of the permeation layer. The mixture was
cast onto the surface of the micro-location. It was
5   then photopolymerized by ultraviolet light. In some
cases, AuCl4 was added as a photoinitiator. The polymer
formulations were cast from water and the nonaqueous
solvents, methanol, tetrahydrofuran, acetonitrile, ace-
tone, and mixtures of these solvents.
10   DNA capture probe was attached to the surface of
the permeation layer by a Schiff base reaction between
an oxidized ribonucleoside attached to the DNA capture
probe and the primary amine of the poly-l-lysine. This
provides evidence of covalent attachment of special
15 functionality to the surface of the permeation layer.
          An oxidized DNA capture probe was brought to a
surface micro-location by electrophoretic transport.
The capture probe was labeled with a fluorescent marker.
This demonstrates the ability to address a micro-loca-
20 tion by electrophoretic transport.
          An oxidized capture probe with a fluorescent marker
attached was attracted to the surface of the permeation
layer at a micro-location by electrophoretic transport.
The permeation layer was removed from the micro-location
25 by mechanical means. No evidence of the presence of the
fluorescently labeled capture probe was observed. This
demonstrates the ability of the permeation layer to
protect the DNA from the electrode surface.
          The maximum DC current density that was attained at
30 a gold micro-location, which was not modified with a
permeation layer, before bubbles due to water hydrolysis
appeared was 8 milliampheres/cm2. The maximum DC cur-
rent density that was attained at a gold micro-location,
which was modified by an acrylamide-based permeation
35 layer, before bubbles due to water hydrolysis appear was
40 milliampheres/cm2. This demonstrates the ability of
the permeation layer to raise the maximum accessible

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cases, the boxes show the programmed current or voltage
as a dotted line, and the measured current or voltage as
a solid line.

In addition to the preferred embodiment of the
5   invention and the alternatives described above, several
more alternatives are possible. For example, the elec-
tric field that gives rise to ion migration may be modu-
lated in time as long as a DC bias voltage or current is
applied simultaneously. The use of an AC signal super-
10   imposed on a DC bias voltage or current can achieve
three things, 1) minimize the background due to nonspec-
ifically bound DNA, 2) provide a means of electronic
stringency control where the control variable is the
frequency of the alternating current or voltage, 3)
15   provide a means of aligning DNA molecules spatially.

Many alternatives to the detection of hybridized
DNA by fluorescence exist. Most of the alternative
techniques also involve modification of capture or tar-
get or reporter DNA probes with reporter groups that
20   produce a detectable signal. A few of these techniques
based on purely physical measurements do not require
reporter groups. These alternative techniques are cata-
logued as follows: (1) Linear Optical Methods including
fluorescence, time modulated fluorescence, fluorescence
25   quenching modulation, polarization selective fluores-
cence, absorption, specular reflectance, changes in
index of refraction, ellipsometry, surface plasmon reso-
nance detection, chemiluminescence, speckle interferome-
try and magneto-optic Kerr effect; (2) Nonlinear Opti-
30   cal Methods including second harmonic generation, third
harmonic generation, parametric mixing, optical heterod-
yne detection, phase conjugation, soliton damping and
optical Kerr effect; (3) Methods Based on Thermal Ef-
fects including differential scanning calorimetry, mult-
35   ifrequency differential scanning calorimetry, and dif-
ferential thermal analysis; (4) Methods Based on Mass
Changes including crystal microbalances, cantilever

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<u>Claims</u>:

1.   An electronic device adapted for performing
molecular biological processes comprising:

         a support having a first generally planar sur-
5   face,

         a plurality of self-addressable electrodes
disposed on the first surface of the support, the
electrodes having a contacting portion adjacent the
first surface of the support, and

10   individual electrical connections to the elec-
trodes.

2.   The electronic device of Claim 1 wherein the
individual electrical connections to the electrodes com-
prise leads disposed on the first surface of the sup-
15   port.

3.   The electronic device of Claim 1 wherein the
individual electrical connections to the electrodes are
electrical pathways extending from the contacting por-
tion of the electrodes into the support.

20   4.   The electronic device of Claim 1 wherein the
support includes an insulator.

5.   The electronic device of Claim 4 wherein the
insulator is an oxide.

6.   The electronic device of Claim 1 wherein the
25   support includes a semiconductive material.

7.   The electronic device of Claim 6 wherein the
support including the semiconductor further includes an
oxide layer disposed thereon.

8.   The electronic device of Claim 6 wherein con-
30   trol electronics are included within the semiconductive

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a controller adapted to receive user input and
provide output including control signals to gener-
ate independent electronic environments at the
electrodes,

5   a input system for receiving user instructions
connected to the input of the controller,

a generator to provide the desired electronic
environment at the electrode, the generator operat-
ing under control of the controller output, and

10   an interface adapted to connect the control
system to the active, programmable electronic sys-
tem.

19.   The control system of Claim 18 wherein the
generator comprises a power supply.

15   20.   The control system of Claim 19 wherein the
power supply is a regulated power supply.

21.   The control system of Claim 18 wherein the
generator comprises a waveform generator.

22.   The control system of Claim 18 wherein the
20   interface comprises a relay system.

23.   The control system of Claim 22 wherein the
interface system includes relays to provide selective
connection from the generator to the electrodes.

24.   The control system of Claim 22 wherein the
25   interface includes relays adapted to change the polarity
of the output of the generator.

25.   The control system of Claim 22 wherein the
interface includes relays to provide selective connec-
tion to either a first level or second level of a sig-
30   nal.

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35. The method of complexity reduction of in a
solution containing DNA Claim 32 wherein the severed
material is removed through electrophoretic force.

36. A system for performing molecular biology
5   reactions comprising:
        an input for receiving a sample containing
    material to be analyzed,
        a sample preparation unit, and
        an active, programmable electronic device
10   including a plurality of separately addressable
    electrodes.

37. The system of Claim 36 wherein the active,
programmable matrix comprises an array of electrodes.

38. The system of Claim 36 wherein the active,
15 programmable electrodes further include an attachment
layer disposed above the electrodes.

39. The system of Claim 38 wherein the attachment
layer includes capture sequences.

40. The system of Claim 36 further including a
20 detector operatively positioned to monitor the active,
programmable device.

41. The system of Claim 40 wherein the detector
comprises an imaging system.

◂   42. The system of Claim 41 wherein the imaging
25 system comprises a CCD camera.

43. The system of Claim 40 further comprising an
analysis system adapted to receive the output of the
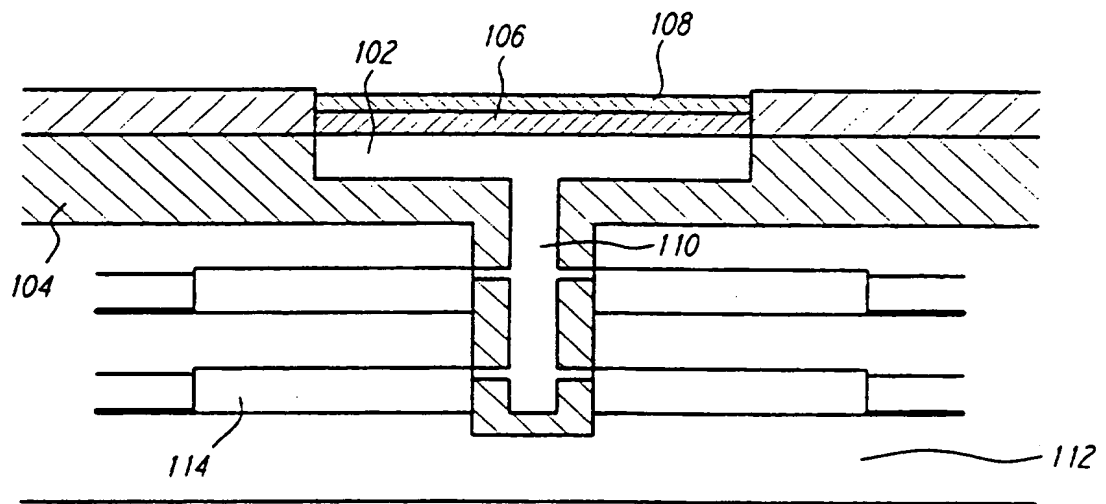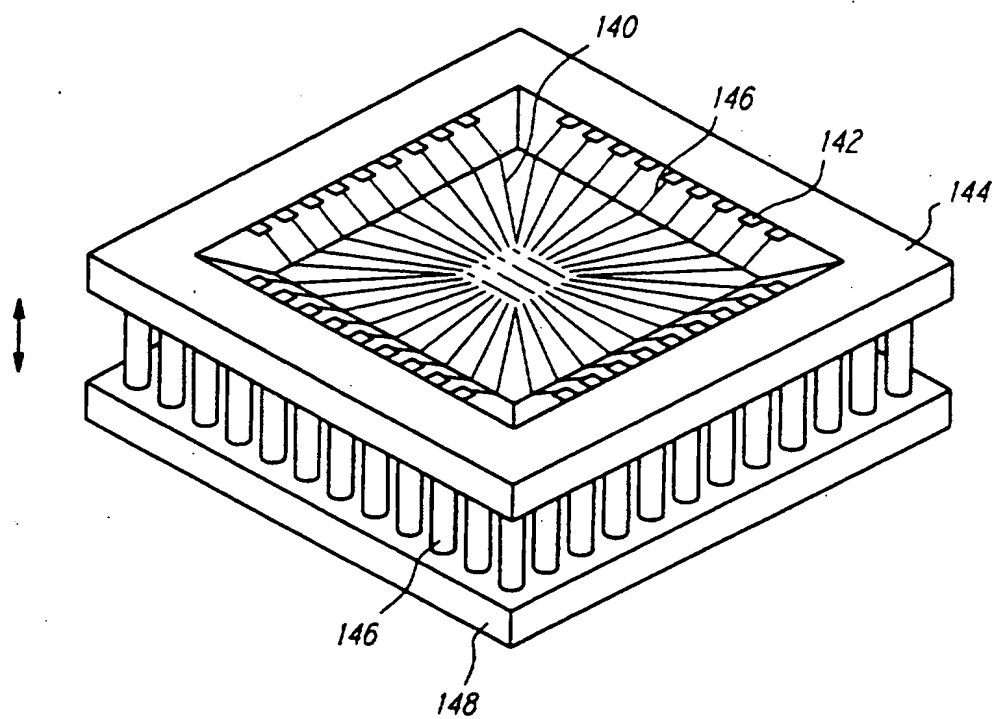detection system.
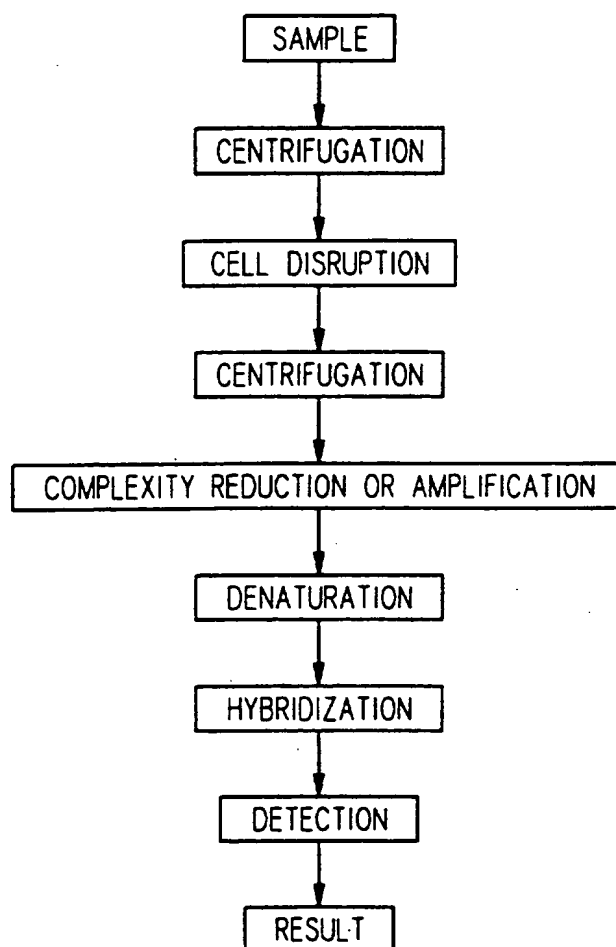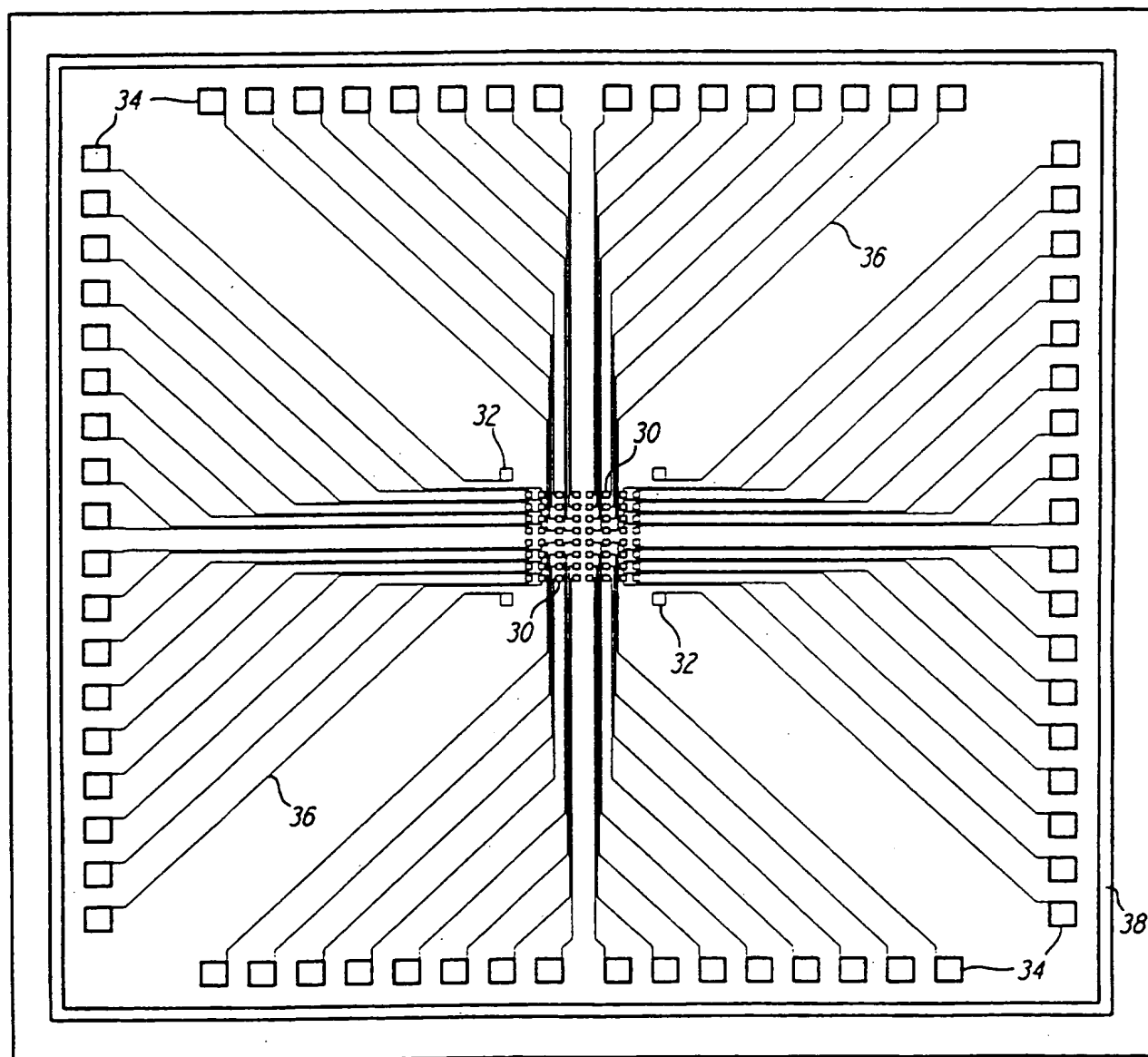
FIG. 1

FIG. 3

FIG. 6

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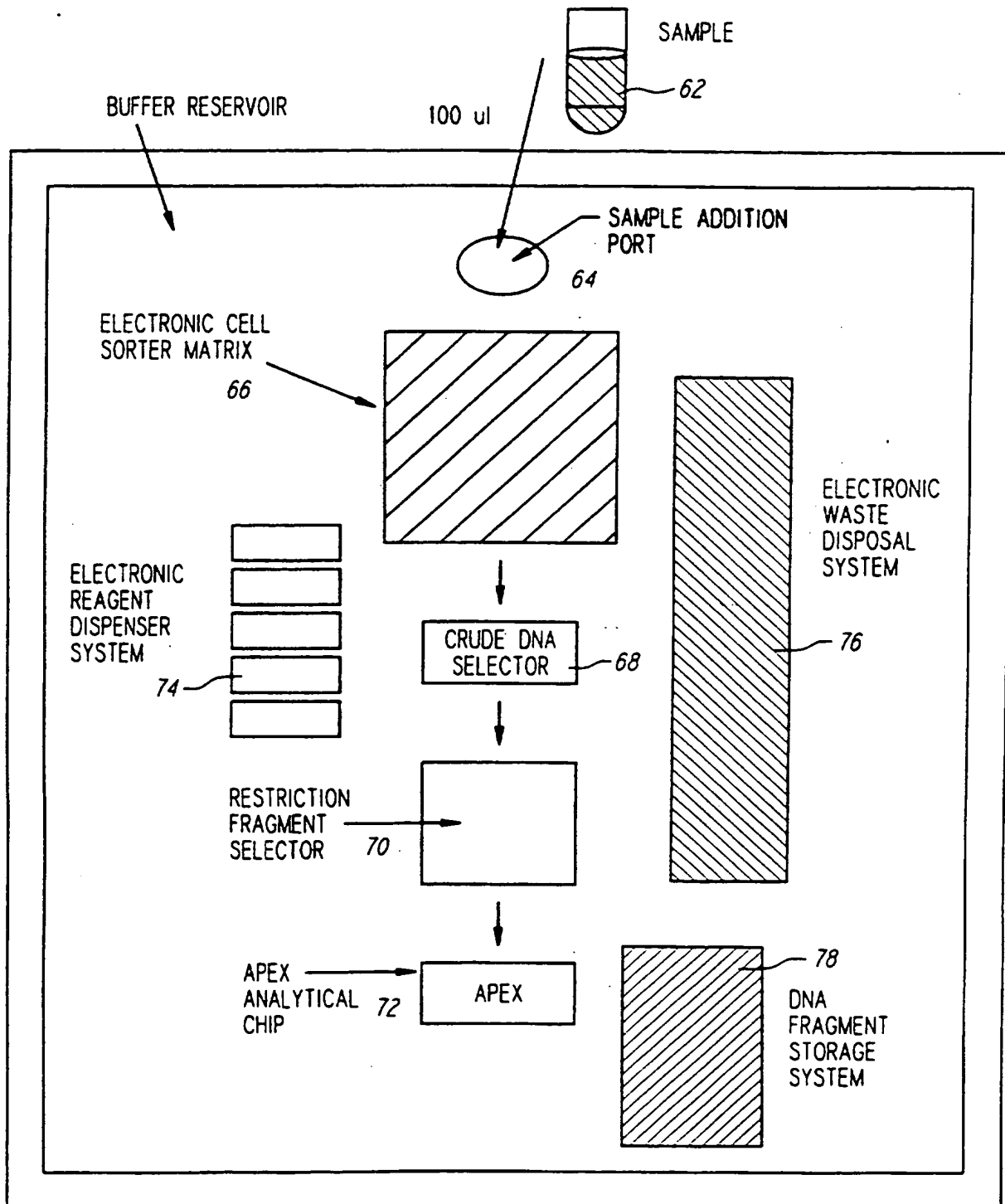
FIG. 8

FIG. 10
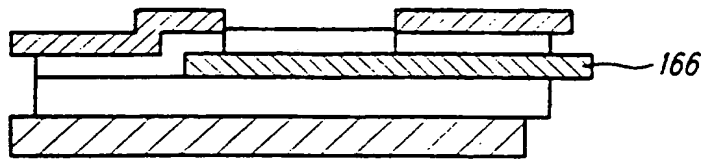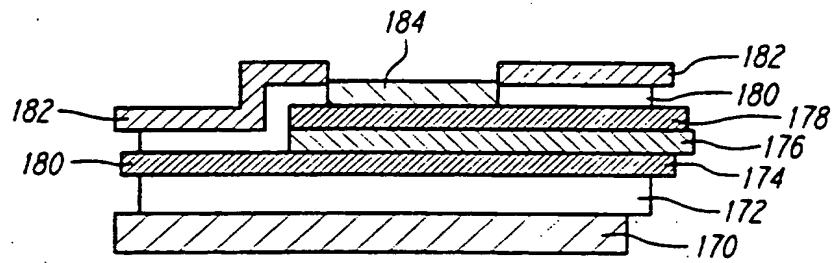
FIG. 12

FIG. 13
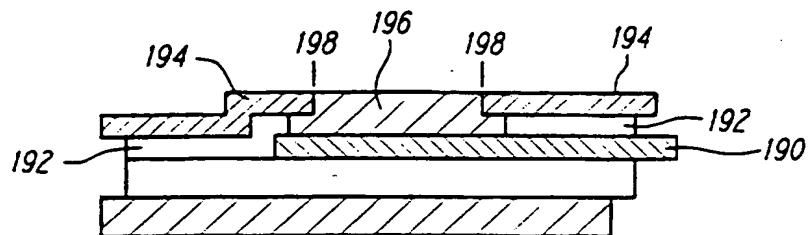
FIG. 14

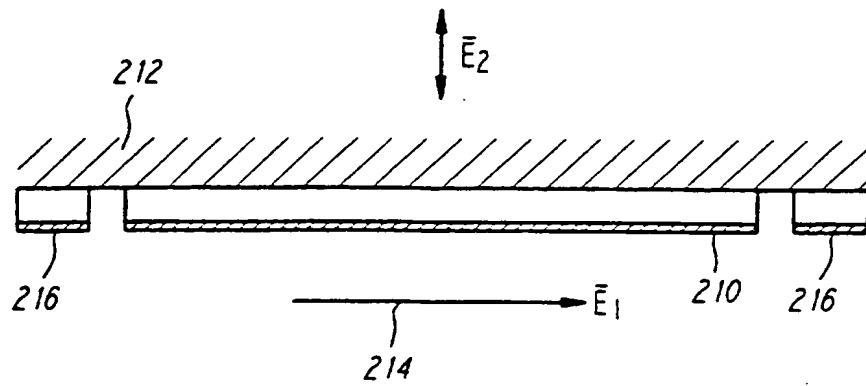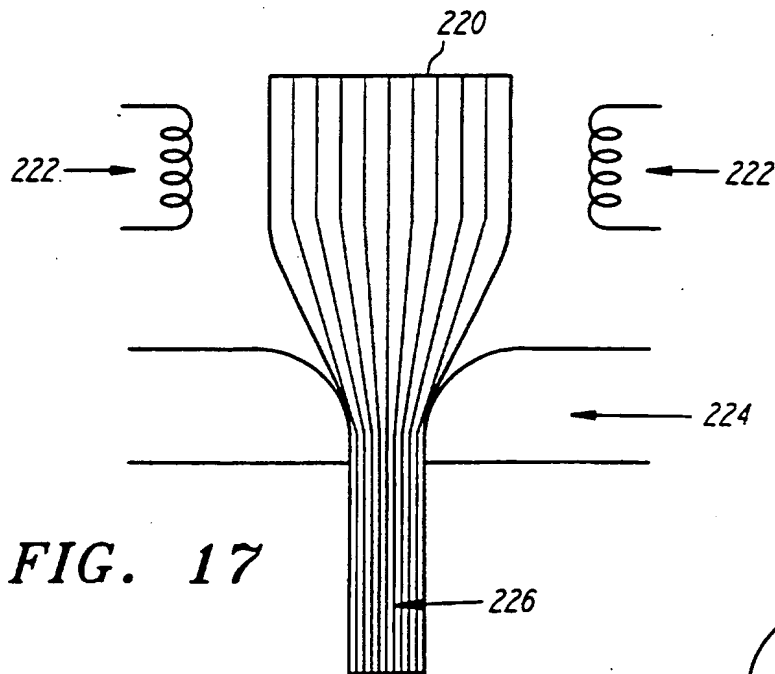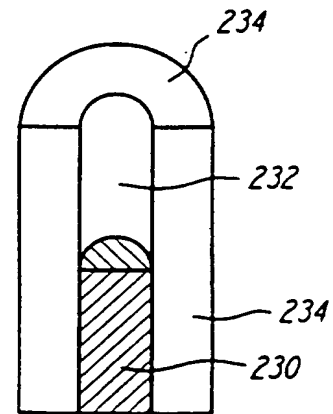*FIG. 16*

FIG. 17

FIG. 18

International application No.

PCT/US95/11333

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US, A, 5,200,051 (COZZETTE ET AL.) 06 APRIL 1993, see especially the abstract; Figures 4 and 8B and related discussion; column 18, lines 1-2; and column 26, lines 8-10.	1-10,13-31,36-40,43, 49-54
		11,12,41, 42,44-48
Y	US, A, 5,096,669 (LAUKS ET AL.) 17 MARCH 1992, see the entire disclosure and especially claims 1-33.	9-13,17-31,36-54
X — Y	US, A, 5,096,807 (LEABACK) 17 MARCH 1992, see especially the abstract, the Figures, and the discussion related to the Figures.	36,40,41, 43
		1-31,37,42,44-54
X — Y	US, A, 5,227,265 (DEBOER ET AL.) 13 JULY 1993, see especially the abstract, the Figures, and the discussion related to the Figures.	36,40,41, 43
		1-31,37,42,44-54
X, P —— Y, P	US, A, 5,304,487 (WILDING ET AL) 19 APRIL 1994, see especially the abstract, Figures, and claims 1-26.	32,36,40, 41,43-49,51,52,54
		1-31,33-35,37-39,42, 50,53